INTRODUCTION

Liver is the largest gland in the body weighing about 1500gms in an adult and accounts for approximately 2.5% of total body weight. Liver is called as the metabolic "engine-room of the body" plays an important vital function in the maintenance, performance and regulating homeostasis of the body and also involved with all the biochemical pathways to growth, fight against disease, supply of nutrient, energy production and storage[1]. Its major influence is on the carbohydrate, fat and protein metabolism, secretion of bile, storage of vitamin and Immunity (the liver contains over half of the body's macrophages). Thus, to maintain a healthy liver is a crucial factor for the overall health and well being[2].

Due to of these high metabolic activity in this organ is an important place for free radicals generation. There are several many enzymes-induced free radicals in the liver, including aldehyde dehydrogenase (ALDH), diamine oxidase (DAO), tryptophan dual oxidase, liver dehydrogenase, and the cytochrome P450 enzyme complex system. Cytochrome P450 enzymes are highly represent in the liver hepatocyte, Kupffer cells, and neutrophils and its responsible for reactive oxygen species (ROS) production[3]. Reactive oxygen species act either positively or negatively on cell functioning based on the intensity and duration of the oxidative stress produced on the cell.

The liver is the central organ for pharmaceutical drug or chemicals and xenobiotic detoxification metabolism, which regulates most of medication and xenobiotic-related toxic activity. Drug or chemical-induced liver damage is a relatively more common major cause of acute or chronic liver disease and carries a mortality rate of around 10%. The metabolic detoxification of drugs and xenobiotics in the liver by drug metabolizing enzymes such as Cytochrome P450, NADPH cytochrome P450 reductase is an important occurrence in the acquisition of homeostasis. Any changes in homeostatic condition leads to a transfer in the dynamic equilibrium of metabolism towards the free radical initiation thereby oxidative stress induce organ dysfunction. Liver damage and inadequacy resulted from exposure to toxicants, certain combinations or overdoses of pharmaceuticals drugs, are main causes of disease and death worldwide[4].

The ideal picture of severe inflammation and damage in the liver is the acute hepatitis caused by different noxae. The adaptation from chronic liver disease to final stages of cirrhosis involves inflammation, stimulate of hepatic stellate cells with initiation of fibrogenesis, angiogenesis, and parenchymal extinction lesions caused by vascular occlusion[5]. This process leads to prominent hepatic microvascular changes, characterized by renovation of sinusoidal (extracellular matrix deposition from proliferating activated stellate cells resulting in capillarisation of hepatic sinusoids), formation of intra hepatic shunts (due to angiogenesis and loss of parenchymal cells), and hepatic endothelial dysfunction[6].

It is thought that inflammation not only precedes but is also need to generate damage of "stressed" hepatocytes. Reversible process of hepatic fibrosis is the common endpoint for most type of chronic liver damage. But it was considered to be an irreversible process, particularly when the complete picture of cirrhosis occurs[7].

Different types of disease may develop different pattern of fibrosis during disease progression[8]. The typical forms of acute viral hepatitis are accompanied by complete recovery of the liver. The hallmarks of chronic infection, caused by hepatotoxic drugs and infection with hepatotropic viruses (hepatitis C and hepatitis B Virus) are inflammation, the death of hepatocytes and finally liver fibrosis[9].

LIVER DISEASE

Throughout the world, liver disorders arise irrespective of age, region, and sex. Chronic liver diseases (CLDs) constitute an important, and certainly underestimated, public health problem all around the world. Global estimations suggesting that 844 million people have CLDs, with a mortality rate of 2 million deaths per year.

Chronic liver inflammation leads to final irreversible stages of liver cirrhosis, which is the 12th leading cause of death in the United State[10], accounting for 32,000 deaths in the US and more than two million deaths each year worldwide[11].

- According to World Health Organization, 35 million people in the world pass way due to acute and chronic liver disease.
- CLDs have been ranked as the 5th main general cause of death as per UK national statistics. According to WHO, liver disease is 10th most common effect of death in India, and it may act every one in five Indians.

- Every year around more than 10 lakh liver cirrhosis patients are newly identified. It is the 14th foremost major cause of deaths in the worldwide and it will become 12th leading cause of deaths in the world by 2020.
- Data published by WHO in May 2019, in India 2.44% or 216,865 of total deaths are due to CLDs. India is ranked 61 in the world. The major effect of liver disease is hepato toxicants, excessive drug therapy, alcoholic intoxication, viruses, and pollution.

STAGES OF LIVER DISEASE

Once the liver comes into contact with viruses or toxins, inflammatory (hepatitis) or fatty (steatosis) changes, or both (steatohepatitis), can occur. At this stage of liver disease, the liver can still regenerate and these changes are usually reversible. If the cause of liver injury is not removed, the damage can progress to fibrosis and finally cirrhosis of the liver.

Healthy Liver

⇓ Reversible Reaction

Inflammation

⇓ Reversible Reaction

Liver Fibrosis

⇓ irreversible Reaction

Cirrhosis

Inflammation

When the liver function is altered due to metabolic or toxic stress, it leads to hepatic steatosis. This steatosis may develop to hepatic damage in response in alcohol (called alcoholic steatohepatitis), hepatotoxins such as carbon tetrachloride and vinyl chloride (called toxicant-associated steatohepatitis), chemotherapy (called chemotherapy-associated steatohepatitis), or metabolic syndrome with insulin resistance (NASH)[12]. Hepatic injury may change hepatocyte gene expression, resulting in increased expression of TGF-β, IL-1A, hedgehog ligands and mesenchymal genes such as twist and snail. The damaged hepatocyte steatosis induces inflammation and it will lead fibrosis**.**

Liver Fibrosis

Hepatic fibrosis is a reversible wound-healing process identified by the deposition of extracellular matrix (ECM) following liver injury and it's initiated by activation of the stellate cells and Kupffer cells, damaged hepatocytes and activated platelets are also involved. At this stage stellate cells and Kupffer cells are released various cytokines such as platelet derived growth factor (PDGF), transforming growth factor β1 (TGF-β1) (potent fibrogenic mediator), tumor necrosis factor (TNF)–α, and their receptors, reactive oxygen intermediates, paracrine signals and autocrine signals.

Inflammatory cells cause fibrosis due to secretion cytokine. Collagens and fibronectin replace the normal matrix in the area of disse. Sub endothelial hepatic fibrosis leads to loss of the endothelial function and reduce liver function. Collagenases (matrix metalloproteinases) are able to degrade collagen but inhibited by endogenous protein, such as tissue inhibitors of metalloproteinases (TIMPs), which level is increased in liver fibrosis.

Liver Cirrhosis

Cirrhosis is end stages of liver disease and characterized by the generation of regenerative nodules in hepatic parenchyma surrounded by fibrous septa due to liver injury. Cirrhosis take place due to necrosis of hepatic cells followed by inflammation, fibrosis and nodule generation. At this stage the structure of liver becomes unusual and inhibit with liver function and blood flow and promote to portal hypertension and reduce hepatocyte's function. CLDs represent a major health problem across the world with liver cirrhosis.

RISK FACTORS

There are various factors that enhance a person 'susceptibility to a potentially hepatotoxic drug. Age, gender, obesity and nutritional status is the risk factor of developing liver disease.

Risk factors for liver disease include the following:

- Genetic factors
- Dose and duration of drugs
- Alcohol and cigarette smoke
- Drug-drug interactions
- Disease such as HIV, hepatitis C

- Kidney damage

In the UK, the most common causes of liver disease are drinking alcohol at harmful levels, non-alcohol-related steatohepatitis due to DM or metabolic syndrome, and chronic infection with either hepatitis B virus or hepatitis C virus.

Some of the less common effect include genetic conditions, autoimmune liver diseases, exposure to certain hepatotoxicant chemicals or medications, and glycogen storage disease.

Drug constituents and drug-drug interaction also may be a reason for high risk of drug-induced hepatotoxicity. Particularly certain pharmaceutical drugs containing a nitro-aromatic moiety or drugs involved with nuclear such as phenobarbital, it may cause organ-selective toxicity or may potentiate the toxicity of other drugs.

ROLE OF CYTOKINES IN LIVER DISEASE

Liver disease is orchestrated by a complex network of cytokine-mediated signaling pathways regulating the hepatic stellate cells (HSCs) and fibrogenesis.

Platelet-Derived Growth Factor (PDGF):

PDGF as most potent factor involved in mitogen to HSCs among all polypeptide growth factors and effectively promotes cell division, proliferation and differentiation[13]. Under physiological condition, PDGF and related receptors are primarily over expressed in fibrous tissues, and activity of these compound increases with the degree of liver fibrosis[14]. A variety of factors such as viruses, chemicals, or mechanical damage to hepatocytes can induce KCs to synthesize and release PDGF[15].

Transforming Growth Factor (TGF)-β:

TGF-β is the strongest known inducer of fibrogenesis in hepatic fibrosis[16]. TGF-β is mainly synthesized by HSCs/myofibroblasts, hepatocytes, and Kupffer cells (KCs) in the liver. The TGF-β1 family is composed of six members, and among them, TGF-β1 has play a major key role in the regulation and maintenance of liver fibrosis[17]. The expression level of TGF-β1 is increased in fibrotic liver and reaches a maximum at cirrhosis[18].

Tumor Necrosis Factor (TNF)-α:

TNF-α is mainly synthesized by monocyte, macrophage, HSCs, and KCs. It has proinflammatory activities and cytotoxic effects in these cells. In the action of

liver fibrosis, TNF-α plays an important major role in regulation of HSCs and production of ECM[19]. TNF-α may decrease the spontaneous apoptosis of initiated HSCs by down regulating the proapoptotic factor p53, as well as upregulating the antiapoptotic factors[20].

Interferon (IFN)

Interferon is a family of soluble extracellular signaling molecules. Leukocytes synthesize two different IFN, such as IFN-α and IFN-β in response to virus infection, and T cells secrete IFN-γ upon stimulation with various type of antigens and mitogens. IFN possess antiviral activity and is well recognized for their antiviral effects[21]. IFN-β could inactivate HSCs and decrease their production of α-smooth muscle actin (SMA) and collagen through inhibition of the TGF-β and PDGF pathways[22].

OXIDATIVE STRESS AND ITS ROLE IN LIVER DISEASES

There is balance between a free radical (FR)/ ROS formation and endogenous antioxidant defense mechanisms, but if this balance is disturbed, it can induce oxidative stress and results in injury to all the important cellular components like protein, nucleic acid and membrane lipid which can cause cell death. Antioxidants have role in protecting the body from the harmful effect of the free radicals[23]. Endogenous antioxidants fight against the ROS are strengthened by natural antioxidants that strengthen them and restore the optimal balance by neutralizing the ROS[24].

The liver metabolizes variety of compounds that produce ROS and generate prooxidants, which can affect tissue liver injury and certain chemical or drugs, external environmental exposures, tissue damage, and infection may can increase prooxidants levels. ROS may can improve from an increase in formation of prooxidant or a decrease or deficiency in antioxidants[25].

Several scientists have given a very close attention to the connection between oxidative stress and chronic diseases because they are of public health importance. Experimental studies are proved that, increasing antioxidant activity is one of the important ways of decreasing oxidative stress. Numerous evidences are supporting the antioxidant activity of Vitamin in reducing the risk of chronic disease by suppressing the state of oxidative stress.

HEPATOTOXIC CHEMICALS

More than 900 chemicals have been suggesting in causing liver injury[26]. Drug caused liver damage is accountable for 5% of all hospital admissions and 50% of all acute liver failures[27]. Drug-induced liver damage is a relatively major common factor of developing acute and chronic liver disease and carries a mortality rate around 10%.

Carbon tetrachloride (CCl4) is one of the experimental xenobiotics drugs that have been reported to promote chronic and acute tissue damage and also its a well-established hepatotoxin used to study in experimental animal models by initiating lipid peroxidation (LPO)[28], thereby causing liver pathogenesis. CCL4 was formerly used for as a dry-cleaning fluid, fabric- spotting fluid and fire-extinguisher fluid and metal degreasing.

CCL4 is expeditiously absorbed by any route of exposure in humans and animals. After absorption, it is widely distributed among all the tissues, particularly those with contain high level of lipid compounds, and reaching peak concentrations level in <1–6 hours, based on exposure concentration or dose. Lung, liver and other tissues can metabolize CCl4 and is rapidly excreted, primarily in exhaled breath.

The principle cause of carbon tetrachloride (CCl4) induced hepatic injury is in LPO and reduce activities of enzymatic and non-enzymatic antioxidants and leads to generation of free radicals.

METABOLIC PATHWAY-CCl4

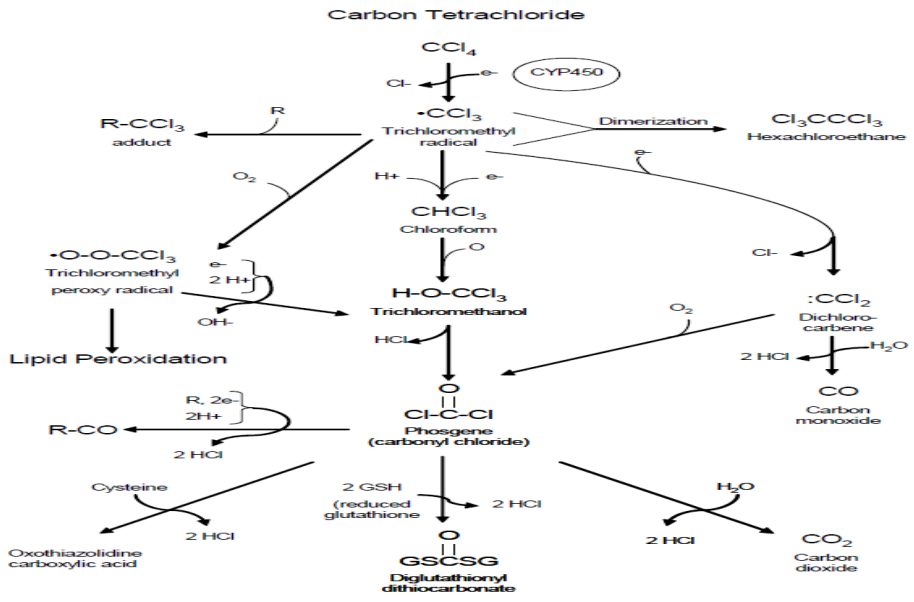

Figure 1: Metabolic Pathway of CCl4

BACKGROUND OF THE STUDY

Early stage of acute and chronic hepatic disease can be prevented by maintaining a healthy diet, lifestyle and avoiding drug and alcohol. It can also prevent by increasing antioxidant and neutralizing the reactive oxygen species. Recent studies are suggesting that, some of vitamins have antioxidant property in lowering the risk of suppressing the state of oxidative stress.

When vitamin D level is adequate, the intracellular oxidative stress related activities are down regulated.

The Vitamin D is a group of sterols compound that have a hormone-like function and it binds with intracellular receptor proteins, Vitamin D receptor (VDR) complex communicates with DNA in the nucleus of target cells and either selectively activate gene expression, or particularly represses gene transcription. Long recognized for its function in regulating calcium–phosphate homeostasis, vitamin D is now involved in an important modulator of the immune response to infection[34] and also been involved in the cell division, proliferation and differentiation mechanisms. It is generally accepted that vitamin D status is related to both innate and adaptive immune system and the multifunctional role of vitamin D is under extensive study.

Bio-Synthesis of Vitamin D

Vitamin D is a secosteroid hormone known for its critical role in calcium and skeletal homeostasis. It is obtained either from dietary sources or synthesised endogenously. In the skin, 7-dehydrocholesterol undergoes a stereoisomeric alteration in response to UV radiation from sun exposure to make pre-vitamin D3, which is transformed into vitamin D3 (cholecalciferol) through non-enzymatic isomerisation (figure 2).

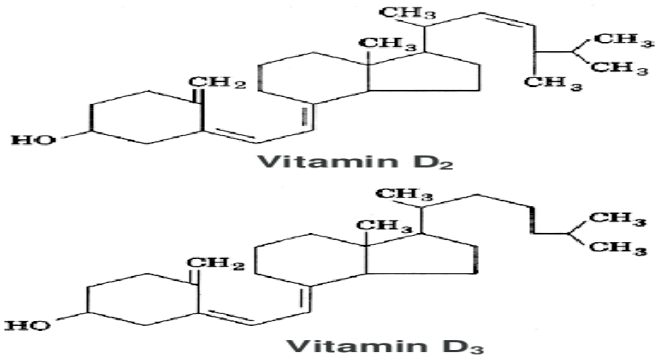

Figure 2: Different forms of Vitamin D

Vitamin D synthesized from skin and from dietary sources may be stored in the adipocyte, or it may undergo 25-hydroxylation in liver. This final process is mediated by isoforms of the P450 cytochrome, the 25-hydroxylases, which produce calcidiol or 25-hydroxyvitamin D [25(OH)D]. This vitamin D substances, like other molecules, it has low-solubility lipophilic molecule that moves via the bloodstream attached to plasma proteins, the most prevalent of which is vitamin D-binding protein (DBP). Up to 88% of serum 25(OH)D is attached to a DBP, a protein synthesized mainly in the liver that has anti-inflammatory and immunomodulating functions independent of its role as a vitamin D transporter[29,30]. 25(OH) D is hydroxylated in the proximal tubules of the kidney by 1α-hydroxylase that form 1α,25(OH)2D or calcitriol, the most biologically active and powerful metabolite of vitaminD[31] (figure 3). 1α-hydroxylase (CYP27B1) activity has been absorbed in the kidney and other organs, including the liver, fat tissue and the innate immune system cells.

The biochemical effects of vitamin D are mediated by binding to the VDR, belongs to the superfamily of nuclear steroid hormone receptors, which is expressed in more than 30 tissues, including the hepatic cells, the pancreatic islet cells, the epithelial cells of the gastrointestinal tract and the immune system cells[32]. Hence, vitamin D deficiency may can be involved in several processes, such as cancer, diabetes mellitus and cardiovascular and autoimmune diseases[33].

VITAMIN D AND CHRONIC LIVER DISEASE

Vitamin D plays a most important role in lowering the risk of chronic diseases, including type 2 Diabetic Mellitus, many types of cancer, and cardiovascular disease, autoimmune and infectious diseases. This role acts through the local synthesis of 25(OH)D and its act has autocrine and paracrine actions in cell division, proliferation and differentiation, apoptosis, renin and insulin secretion and interleukin (IL) and bactericidal protein production[34,35]. These effects may also be relevant in the abnormality of chronic liver diseases.

Severe liver disease reduces vitamin D hydroxylation and albumin and DBP production, all of which are linked to low levels of 25(OH)D. Nevertheless, the vitamin D insufficiency in CLDs is only partly the result of a synthesis dysfunction of the liver, as evidenced by the fact that vitamin D deficiency is highly prevalent in non-cirrhotic patients[36].

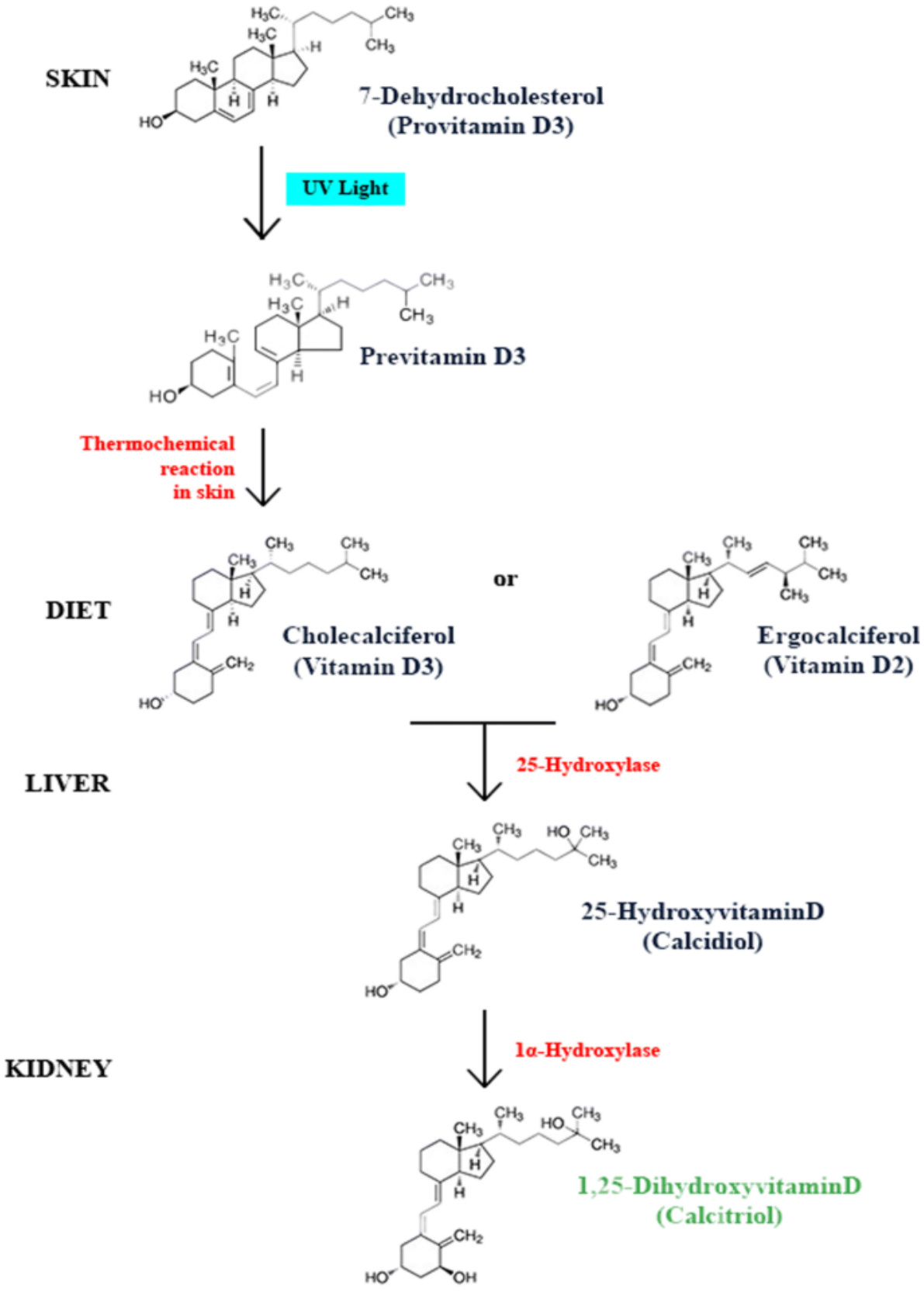

Figure 3: Synthesis of Vitamin D

The levels of 25(OH)D in cirrhotic patients normalize after vitamin D treatment, which indicates that the 25-hydroxylation is pre-served[37,38]; and although DBP is moderately decreased in cirrhosis[39], vitamin D metabolites need only 5% of the DBP binding sites[40], indicating that liver dysfunction must be severe to reduce the DBP levels and contribute to vitamin D deficiency.

Therefore, vitamin D insufficiency in CLDs requires various causes in addition to those pointed above, including insufficient food intake, inadequate sun exposure, steroid use, jaundice related degradation of vitamin production on the skin and reduce absorption of vitamin D effect by portal hypertension and intestinal edema or to cholestasis induced bile salt disruption.

Vitamin D is playing an important role in the decrease of inflammation and fibrosis[41]. Proinflammatory signals in monocytes and macrophages may regulate the local metabolic synthesis of vitamin D, auto-inducing the expression of CYP27B1 and the local production of 25(OH)D, and thus controlling the excessive inflammatory response[42]. Almost ninety percentage of macrophages are present in the liver hepatocyte[43], which indicates that the hepatic production of 25(OH)D is reduced during the inflammatory diseases of the liver. Furthermore, high levels of VDR are present in both macrophages and biliary epithelial cells and other non-parenchymal cells[44]. After activation, these cells increase the expression of cathelicidin, an antimicrobial peptide with anti-endotoxin activity[45], and inhibits the synthesis of biliary acids, thus protecting the hepatocytes from these acids[46]. Therefore, the connection between vitamin D and liver physiopathology may result from signaling disruptions in extrahepatic cells or non-parenchymal liver cells.

Vitamin D Receptor and Chronic Liver Disease

The biological actions of vitamin D are mainly mediated by VDR that belongs to the nuclear receptor superfamily. Upon 25(OH)D binding, VDR appear a heterodimer complex with the retinoid X receptor (RXR) and linked with regions of the DNA called vitamin D response elements (VDRE). These elements important coactivators able to regulate the transcription of target genes involved not only in calcium and phosphate homeostasis[47] but also in cellular proliferation, differentiation and immune system cells[48]. The immune system cells, including dendritic cells, macrophages, and B and T lymphocytes, express CYP27A1 or CYP27B1 enzymes

and thus can metabolize 25(OH) D to calcitriol. Calcitriol will then have an autocrine or paracrine function. Two of the important genes that are activated by VDR such as, anti-ageing gene Klotho and Nrf2, both of which have different roles in maintaining the integrity of cellular signaling systems. In addition, vitamin D combined together with Nrf2 and Klotho all increase cellular antioxidants to maintain the normal reducing environment within the cell.

VDR is highly expressed in the non-parenchymal cells of the liver. Kupffer cells (KCs), sinusoidal endothelial cells and especially HSCs all express VDR. In regards to CLDs, HSCs are the major effector cells in the wound healing process, which is leading to liver fibrosis, being the important source of both physiological and pathological hepatic extracellular matrix. This cell-specific appearance pattern of VDR show that the liver could be vitamin D responsive mediated via its non-parenchymal constituents. Many studies are suggesting that, activation of VDR in HSCs has recently been shown to strongly antagonize TGFβ signaling, the most potent pro-fibro genic pathway in liver, resulting in suppression of a broad range of pro-fibrotic genes including collagens, integrins and tissue inhibitors of metalloproteinase (TIMPs)[49].

Role of Vitamin D in Immune System

The immune system is crucial and essential for human health and survival. With a sub optimally functioning immune system, even minor pathogens may prove fatal. Successful immunity needs a complex of cells and effector biomolecules working together against foreign antigens in day to day with microorganisms and pathogens. These cellular elements are thymus derived T-lymphocytes and bone marrow derived B-cells, that form part of the adaptive immune response and dendritic cells (DCs), macrophages, natural killer (NK) cells and granulocytes which compose the innate arm. In response to foreign or unknown antigens whatever their origins (e.g., cancers, pathogens, and vaccines), a number of cytokines like interleukin (IL) 1, IL-6, tumor necrosis factor α (TNFα), and interferon γ (INFγ) are released by the cellular component[50].

The immune system cells, including macrophages, dendritic cells, and T and B lymphocytes, expressCYP27A1 or CYP27B1 enzymes and thus can metabolize25(OH)D to calcitriol. Calcitriol will then have an autocrine or paracrine function. Vitamin D favours the innate response of the immune system and has a

“self-regulatory” effect by limiting the adaptive response. On one hand, it stimulates the synthesis of antimicrobialpeptides (cathelicidin and beta-defensin) and the chemotaxis and phagocytosis of the macrophages. On the other hand, it decreases the expression of class II complex molecules, co-stimulating molecules and the synthesis of Th1, Th2 and Th17 cytokines. Finally, in addition to acting as a transcription factor, VDR seems to induce fast non-genomic responses by activating cellular signalling pathways.

Toll-like receptors (TLRs) are important components of the innate immune response, elicit direct antimicrobial activity against intracellular bacteria such as MTb. TLRs are regulated by macrophages through up-regulated expression of the VDR and the vitamin D hydroxylase genes leading to the induction of the antimicrobial peptide like cathelicidin, which kills intracellular MTb. Moreover, 25 (OH)D boosts infection-stimulated cytokine/chemokine responses, including interleukin 1β (IL-1β) expression, by a direct transcriptional mechanism[50].

25 (OH) D induces the modification of native T cells to regulatory T cells via paracrine effects on CYP27B1/VDR-expressing DCs[51]. The main effect of 25 (OH) D on T cells appears to be the formation and differentiation of T helper type 1 (Th1) and T helper type 2 (Th2) cells, with 25 (OH) D favoring Th2. The balance between T helper type1 and 2 has important significant for the immune response to inflammation. Pro-inflammatory cytokines such as interferon gamma (INF-γ) synthesis from Th1, whereas anti-inflammatory cytokines such as IL-4 and IL-5 are synthesis from Th2 cells. In promoting Th2 development, vitamin D thereby limits tissue damage from the inflammatory response[52].

Current evidence suggesting, that the circulating level of 25(OH)D may be crucial for the optimal anti-inflammatory response of human monocytes[53]. The metabolic changes of 25(OH)D to its active form 1,25(OH)2 occurs locally in immune system cells. The active metabolite of vitamin D has an anti-inflammatory effect on the inflammatory profile of monocytes, down-regulating the expression and production of several pro-inflammatory cytokines including TNF- α, IL-1β, IL-6, and IL-8[54]. Some quick vitamin D action take place in cells that possess the membrane vitamin D receptor (mVDR). However, the majority of vitamin D’s biochemical functions are mediated via the activation of gene expression. The active molecules of 1,25 dihydroxy vitamin D binds to its nuclear vitamin D receptor (nVDR) with high affinity and specificity. The vitamin D-nVDR forms a heterodimer with the RXR and

this complex represses transcription of the target genes via its binding to vitamin D responsive elements on DNA[55]. The nVDR is found in almost all cells of the immune system such as human neutrophils, B cells, macrophages, dendritic cells, and Treg cells[56].

Recently several other experimental clinical studies point towards a direct major role of vitamin D in modulating hepatic inflammation and fibrogenesis and increasing hepatic response, likely through the binding to specific VDR, which is expressed on different cell types into the liver. Vitamin D is involved in the decrease of inflammation and fibrosis. Once proinflammatory is activated it transfer signals in monocytes and macrophages to regulate the local metabolism of vitamin D, auto-inducing the expression of CYP27B1 and the local production of 1α,25(OH)2D, and thus controlling the excessive inflammatory response. Liver tissue contains 90% of macrophages, which indicates that the liver manufacture of active vitamin D is affected during inflammatory diseases of the liver. Therefore, active form of 25(OH)D and 1,25(OH)2D modulate monocytes and macrophages, and DCs activation in terms of VDR, CYP27B1, (the enzyme degrading 1,25(OH)2D), production of soluble mediators, proliferation and maturation, and the expression of cell membrane receptors and through endocrine, paracrine, and intracrine mechanisms[57].

Role of Vitamin D in Oxidative Stress

1,25(OH)2D is involved in several biochemical enzymatic reactions, and intracellular genomic activities and since 25(OH)D concentrations are important major role in overcoming inflammation, the reduction of oxidative stress following the day-to-day exposure to toxic agents, and controlling the aging process[58]. For example, the presence of a physiologic 25(OH)D concentration enhances the expression of the nuclear factor, erythroid-2(Nf-E2)-related factor 2(Nrf2)[59] and enhances Klotho, a phosphate regulating hormone and also an antiaging protein[60]. It also facilitates protein stabilization. Klotho also regulates cellular signalling systems, including the formation of antioxidants[61].

When vitamin D level is adequate, the intracellular oxidative stress related activities are down regulated. Insignificant concentrations of serum vitamin D fail to subdue oxidative stress conditions, augment intracellular oxidative damage and the rate of apoptosis. The intracellular level of Nrf2 is inversely correlated with the

accumulation of mitochondrial ROS[58]and the consequents calation of oxidative stress. Thus, Nrf2 plays a key role in protecting cells against oxidative stress; this is modulated by vitamin D[62]. In addition, vitamin D supports intracellular oxidation and reduction (redox) control by maintaining normal mitochondrial functions[63,64]. Loss in the redox control of the cell cycle may lead to aberrant cell proliferation, cell death, the development of neuro degenerative diseases. Calcitriol has overarching beneficial effects in upregulating the expression of certain antioxidants and anti-inflammatory cytokines[65], thereby protecting the tissues from toxins, micronutrient deficiency-related abnormalities, and parasitic and intracellular microbe-induced harm[66]. It regulates ROS levels through its anti-inflammatory effects and mitochondrial-based expression of antioxidants through cell-signalling pathways[67].

Vitamin D as well upregulates the expression of Gpx that converts the ROS molecule H2O2 to water[68]. Vitamin D also effect the generation of glutathione via activation of the enzyme glucose-6-phosphate dehydrogenase[68] which downregulates nitrogen oxide, a strong precursor for generating ROS that converts $O2^-$ to H2O2 and upregulating superoxide dismutase (SOD). These vitamin D-related actions collectively reduce the burden of intracellular ROS.

Role of Vitamin D in Neutralization of Toxins

Vitamin D and VDR interaction, the transcription factor Nrf2 translocates from the cytoplasm to the nucleus. Nrf2 activates the expression of several genes that have antioxidant activity[69]. When Nrf2 activity is insufficient, risks from oxidative stress-related tissue damage increases[70]. The excessive ROS generation by dysregulated mitochondria leads to a pathologic oxidative stress, a key important cause of toxin-induced liver disease. Meanwhile, the Nrf2 activity in part is controlled by the cytosolic protein Keap1[71], another transcription factor and a negative regulator of Nrf2. Keap1 also controls the sub cellular distribution of Nrf2 that correlates with its antioxidant activity[69]. When confronted with intracellular oxidative stresses, as a protective mechanism, the Nrf2-antioxidant response path is activated. This response increase gene transcription and translation of protein products that are necessary to eliminate or neutralize toxins, and ROS.

LIV-52

The domestic system of medicine in India has a long tradition history of treating liver disorders with plant drugs. Liv.52 is one such important polyherbal proprietary formulations. Herbal product of Liv.52 is approved by the Government of India's through Drug Regulatory Authority, Yoga, Naturopathy, the department of Ayurveda, Unani, Siddha and Homoeopathy of the Ministry of Health and Family Welfare. The effectiveness of Liv.52 in liver disorders of various etiologies is confirmed by a significant number of clinical trials conducted across the world. It is also beneficial in treating viral hepatitis, drug-induced hepatic damage, and alcoholic liverdisorders[72].

Liv.52 is an herbal hepatoprotective formulation discovered in 1955 by Himalaya Herbal Health Care Company, since then, it has been sold global level and has been accepted by thousands of health professionals.

The natural compounds in Liv.52 exhibit potent hepatoprotective properties against drug or chemically-induced hepatotoxicity. It replaces the functional efficiency of the liver by protecting the hepatic parenchyma cells and promoting hepatocellular regeneration. The antioxidative activity of Liv.52 prevents the loss of functional integrity of the cell membrane, maintains cytochrome P-450 enzyme system[73]. Liv.52 is known to improve the functional efficiency of the liver by promoting detoxification and thus protecting from harmful food and medication toxins, maintaining healthy levels of liver enzymes. Liv.52 is also known to support liver's normal ability to burn fat and maintain body's metabolic homeostasis.

AIM AND SCOPE OF THE STUDY

Chronic liver diseases are growing public menace and more than two million new case of this disease are reported every year. Carbon tetrachloride frequently used as a fabric-spotting fluid, dry-cleaning agent, reagent in chemical synthesis, solvent, and fire extinguisher fluid. Therefore, people can rapidly absorb CCl4 by any route of exposure that are more prone to develop liver disease. The aim of the present study has been to overcome these problems and to find out an alternative drug. The combination therapy of 25- Hydroxycholecalciferol and Liv-52 have protective role and also the protective role of 25-Hydroxycholecalciferol in isolation, in assessing the carbon tetrachloride induced liver disease.

The principal objectives of the study include

- ✓ To evaluate the combination therapy of 25 Hydroxycholecalciferol and Liv-52 against Carbon tetrachroride (CCl4) induced liver disease in experimental rats.
- ✓ To evaluate the antioxidant properties of 25 Hydroxycholecalciferol and Liv-52 against carbon tetrachloride (CCl4) induced liver disease in experimental rats.
- ✓ To evaluate the free radical scavenging activities of 25 Hydroxycholecalciferol and Liv-52 against carbon tetrachloride (CCl4) induced liver disease in experimental rats.
- ✓ To assess the metabolic enzyme and liver markers alterations of 25 Hydroxycholecalciferol and Liv-52 CCl4 induced liver disease in experimental rats.
- ✓ To evaluate lipid peroxidation and lipid profile levels of 25 Hydroxycholecalciferol and Liv-52 against carbon tetrachloride (CCl4) induced liver disease in experimental rats.

MATERIALS AND METHODS

Animals care and housing

Adult male Albino Wistar rats (150-200 gms) were purchased from Biogen Laboratory Animal Facility, Bangalore, Karnataka and housed in Meenakshi medical college and Research institute, Kanchipuram, Tamilnadu. The animal was housed in polypropylene cages (24 x 14 cm and 6 rats per cage) and maintained in a controlled environmental condition of temperature (23±2ºC) and relative humidity (50-70%) on alternatively 12 hr light/dark cycles. All animals were fed standard pellet diet (Gold Mohor rat feed, M/s. Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The rats were acclimatized to laboratory condition for one week before commencement of the

experiment. This research work on Wistar rats was obtained and approved by the Institutional Animal Ethical Committee (REG No. 765/03/ca/CPCEA).

Experimental Protocol

The animals were divided into eight groups and each group consists of six animals.

Group I : Normal control

Group II : CCl4 treated animals (1 mL/kg b.w., 50% CCL4 in olive oil) twice a week for five weeks.

Group III : CCl4 + Treated with Vitamin D (500 IU/kg. body weight) daily for five weeks.

Group IV : CCl4 + Treated with Liv-52 (1 mL/kg. body weight) daily for five weeks.

Group V : CCl4 + Treated with both Vitamin D and Liv-52 (as above).

Group VI : Control animals treated with Vitamin D (500 IU/kg. body weight) daily for five weeks.

Group VII : Control animals treated with Liv-52 (1 mL/kg. body weight) daily for five weeks.

Group VIII : Control animals treated with both Vitamin D and Liv-52 (as above) daily for five weeks.

After the experimental period of five weeks, all the animals were food deprivation overnight and anesthetized by exposing to diethyl ether and then sacrificed by cervical decapitation. 2 ml of the blood were collected from all the rats through the tail and retro-orbital venous plexus under ether-induced anaesthesia, into plain dry test tube (without anticoagulant) and supernatant was separated and used for biochemical assays. Liver tissue was immediately dissected out and washed in saline and patted dry and weighed. Around 100 mg tissue from liver was taken and homogenized (10% w/v) with 0.1M Tris-HCL buffer in ice cold condition. The

supernatants were separated, stored at 4ºC for one week and used for biochemical and histopathological assay.

- Histopathological study of liver tissue was carried out to identify the pathological changes in CCl4 induced, drug treated and control groups.
- Changes of body weight of the experimental animals were monitored daily.

Furthermore, the following biochemical parameter were also carried out.

S.No	Parameters	Method
1.	Estimation of Superoxide dismutase (SOD)	Superoxide Dismutase (Superoxide: Superoxide oxidoreductase) activity was estimated by using the method of Marklund (1974).
2.	Estimation of Catalase (CAT)	Catalase activity was estimated by using the method of Sinha (1972).
3.	Estimation of Glutathione peroxidase (GPx)	Glutathione peroxidase (Glutathione: Hydrogen peroxide oxidoreductase) activity was estimated by using the methods of Rotruck*et al*.(1973) and Beutler*et al*. (1963).
4.	Estimation of Reduced glutathione (GSH)	Reduced Glutathione content was estimated by using the method of Ellman (1959) with little modification (Beutler*et al*., 1963).
5.	Estimation of Hydrogen peroxide (H2O2)	The ability of extracts to scavenge H2O2 was determined according to the method of Ruch *et al*. (1989).
6.	Estimation of Superoxide anion(O2)	Superoxide anion radical scavenging activity was estimated by the method of Liu *et al*., (1997).
7.	Estimation of Hydroxyl radical (OH)	Hydroxyl radical scavenging activity was estimated according to the modified method of Halliwell *et al*., (1987).
8.	Estimation of Lipid peroxidation	The level of lipid peroxides was assayed by using the method of Ohkawa*et al.*,(1979).
9.	Estimation of Alkaline phosphatase (ALP)	The level of ALP was assayed by using the method of Powell *et al.*,(1954)

10.	Estimation of Alanine amino transferase (ALT)	The level of ALT was assayed by using the method of Wilkinson*et al.*,(1972)
11.	Estimation of Aspartate amino transferase (AST)	The level of AST was assayed by using the method of Wilkinson*et al.*,(1972)
12.	Estimation of γ-GT	The activity of γ-glutamyl transpeptidase was estimated according to the method of Orlowski and Meister (1965) modified by Rosalki and Rao (1972).
13.	Estimation of Total protein	The level of Total Protein was assayed by using the method of Michael *et al.*,(1978)
14.	Estimation of Albumin	The level of albumin was assayed by using the method of Harding and Keyser, (1968) (Bromocresol Green)
15.	Estimation of Calcium	The level of Calcium was assayed by using the method of Baron and Bell (1958)
16.	Estimation of Phosphorus	The level of Phosphorus was assayed by using the method of Fiske-Subbarow (1956)
17.	Estimation of Total cholesterol	Serum Total cholesterol was estimated by using the method of Parekh and Jung (1970).
18.	Estimation of Free cholesterol	Free cholesterol was estimated by using the method of Leffler and McDougald (1963).
19.	Estimation of Ester cholesterol	Ester cholesterol was measured by using substraction of free cholesterol come total cholesterol.
20.	Estimation of Phospholipids	Phospholipids was estimated by using the method of Rouser *et al.* (1970)
21.	Estimation of Triglycerides	Triglycerides were estimated by using the method of Rice (1970) based on using the method of Vanhandel (1961).
22.	Estimation of Free fatty acids	Free fatty acid content was estimated by using the method of Hron and Menahan (1981).
23.	Estimation of Vitamin D	25 (OH) D were estimated by using the method of Bikle (2013) based on using the method of Van Hooff (1998)

CONCLUSION

In the present study indicate that 25-Hydroxycholecalciferol and Liv-52 had a protective effect against CCl4-induced hepatic damage in experimental rats. The level of liver marker enzymes were increased in CCl4 induced rats and it decreased treatment with combination of both vitamin D and liv-52. Levels of enzymatic and non-enzymatic antioxidants were decreased in CCl4 induced rats and upon combination treatment these levels were increased. The free radical scavenging activities were increased in CCl4 induced rats and treatment with vitamin D and liv-52 decreased these activities. The results of the present study will be discussed comprehensively in the thesis.

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